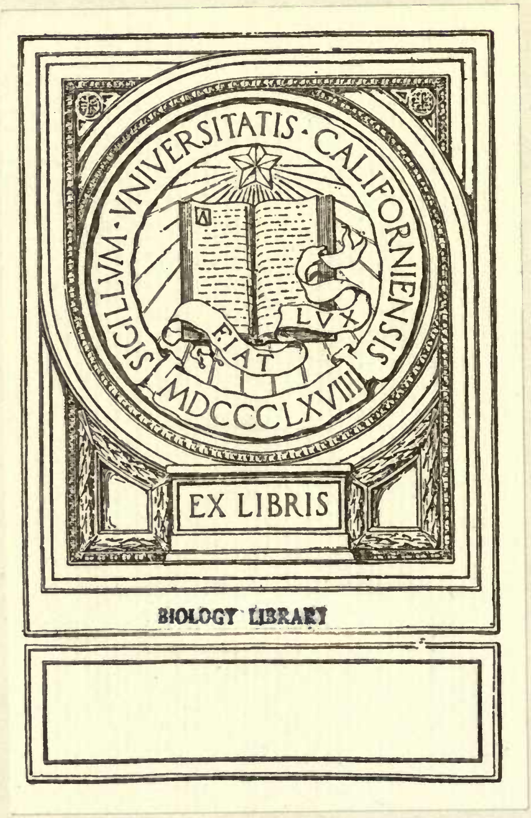
QL
863
K6

UC-NRLF
$C 100 817

1906

~~Property of~~
~~The Department of Anatomy~~
~~Reserve Book Shelf~~



BIOLOGY LIBRARY

ON THE NATURE OF THE GRANULE CELLS OF PANETH IN THE INTESTINAL GLANDS OF MAMMALS

BY

SIDNEY KLEIN, S. M., M. D.

From the Hull Laboratory of Anatomy, University of Chicago

WITH 5 FIGURES

Reprinted from THE AMERICAN JOURNAL OF ANATOMY, Vol. V, No. 3, pages 315-330

JULY 25, 1906

K 6

BIOLOGY
LIBRARY

ON THE NATURE OF THE GRANULE CELLS OF PANETH IN THE INTESTINAL GLANDS OF MAMMALS.

BY

SIDNEY KLEIN, S. M., M. D.

From the Hull Laboratory of Anatomy, University of Chicago.

WITH 5 FIGURES.

The recent activity in the investigation of the chemical and physiological properties of the succus entericus, and the discoveries of new enzymes which are produced by the intestinal mucous membrane, create a renewed interest in the structure and relationship of the elements composing the intestinal epithelium and glands, which are the sources of this secretion. It becomes a fundamental problem of intestinal histology to determine as far as possible the cytological and microchemical characters of these elements and to compare them in these respects with similar elements of known function from other sources. Of special interest in this connection are the peculiar, coarsely granular cells which occupy the deeper ends of the glands of Lieberkühn, and which were first observed in 1872 by Schwalbe, **72**, in fresh material from the intestine of the rat.

For some reason Schwalbe's description attracted little attention, and it was not until 1888 when Paneth, **88**, rediscovered them and described at some length their microscopic and chemical characters, that these cells became generally recognized as constant constituents of the intestinal glands of certain mammals. For this reason they are generally known as the granule cells of Paneth.

Paneth regarded these granular cells as a specific kind of gland cell wholly different from the globlet cell. Concerning their origin he was somewhat in doubt, although he favored the view that they were derived from indifferent mitotic cells farther up the gland, because of the fact that the granules became fewer towards the middle of the gland where the mitoses occurred.

Paneth described the granules, as observed in fresh preparations of the mucous membrane, as moderately refractive structures, although not so refractive as fat. Distilled water and solutions of caustic potash had no effect on them, although they shrank somewhat in the latter and became

737104

more refractive. Ether and alcohol on the other hand dissolved the granules slowly and in diluted acids they disappeared instantly. By means of osmic acid and of picric acid he succeeded in fixing the granules and the cells which contained them. Flemming's fluid gave unsatisfactory results. He regarded the cells in question as a special kind of glandular cell different from the goblet cell. Concerning the fate of these cells Paneth did not express himself definitely although he inclined to the view that they are completely used up in secretion and are replaced by mitotic cells farther up the gland. His attempts to show that this was the case by observing the effect of physiological stimulation, however, did not lead to the desired result, inasmuch as the cells were as numerous in the animals which had been fed as in those which were examined in a state of hunger.

Nicolas, 91, also studied the granule cells and described at considerable length the different varieties of cells to be found in the bottom of the gland of Lieberkühn. Some of these he regarded as secretory phases in the history of the cell of Paneth. Of these he recognized several, of which the following may be mentioned: (a) indifferent cells with clear protoplasm; (b) fine granulations appear in the protoplasm-primary granules; (c) the granules contain a safraninophilous body in the form of a crescent or semicircle, the rest of the granule staining in Flemming's fluid; (d) the secretory activity has attained its maximum and the cell is completely filled with granules containing a safraninophilous body; (e) the cells expel the granules; (f) the cell contracting after expelling its contents assumes the aspect of the small narrow cell with deeply stained protoplasm; (g) the cell recovers itself and assumes the appearance of stage *a*. Nicolas also observed that in the later stages of secretion the nucleus became smaller, often irregular in shape, and stained diffusely, whence he concluded that the nucleus participated in the secretory activity of the cell.

Bizzozero, 93, did not acept the conclusion of Paneth and Nicolas that the granule cells were specific glandular elements, but attempted rather to bring the facts with regard to them into accord with his theory that the glands of Lieberkühn were not in reality true glands, but merely foci for the regeneration of the surface epithelium, and to convert the apparently adverse fact of the occurrence of peculiarly organized elements in the bottom of the glands into an additional proof of the validity of his theory. He claimed to have found, in material from the intestine of the mouse stained in safranin and hæmatoxylin after fixation in Hermanns' fluid, what he considered to be transitional forms between Paneth cells and goblet cells. In these preparations the mucin in the goblet cells was stained a violet color while the Paneth granules became red. The cells which were regarded by Bizzozero as transitional cells contained both small red granules and large blue mucin granules. As these cells were observed in an intermediate position in the gland between the Paneth cells below and the goblet cells above it seemed probable to Bizzozero that the mucin in them had been produced by the transformation of Paneth granules. He assumed, therefore, that the Paneth cells were young goblet cells.

Subsequent investigators, however, among whom may be mentioned Möller, 99, Zimmermann, 98, Zipkin, 04, and Schmidt, 05, have failed to find the transitional elements described by Bizzozero, and have accepted the conclusion of Paneth and Nicolas that the granule-cells are specific elements engaged in a special kind of secretion.

Schaffer, 91, described and figured these structures in the glands of Lieberkühn of the duodenum and jejunum of man although he did not succeed in staining the granules. Zimmerman found that the granules stained strongly in iron hæmatoxylin in sections of human small intestine fixed in sublimate. He regarded the cells of Paneth as serous cells.

Möller, 99, studied the structure of the glands of Lieberkühn of a large number of mammals, chiefly in material fixed in a formaldehyde bichromate mixture, and stained in the Ehrlich-Biondi mixture, although he also used other fixing fluids for purposes of control, and applied the iron-hæmatoxylin method with good results. Möller found that the cells of Paneth occurred in the intestinal glands of the mouse, guinea-pig, rabbit, ox, sheep, and horse. His results were negative as regards the cells of Paneth in the pig, cat, and dog, although he regarded the failure to find them in the first-named animal as due to a failure to fix the granules. Möller also found that the granules in different cells often exhibited different affinities for the stains employed, so that, for example, in sections stained in the Biondi-Ehrlich mixture some granules stained red, others yellow, greenish-yellow, or dark olive green. This difference he thought to be due to different functional conditions of the cells, the changes which the granule underwent from the time of its first formation in the cell to the time when it reached its mature form being indicated by its staining properties. In some cells he found indications of the fusion of the separate granules to a common mass which in part occupied the meshes of the cell framework, in part the wide lumen of the gland. These facts he regarded as undoubted indications of a real secretory activity on the part of these cells. He found no transitions between Paneth cells and goblet cells.

Zipkin, 04, describes the Paneth cells of Inuus rhesus as present without exception in the bottom of every crypt, often lying beside one another in considerable numbers. The protoplasm of these cells always stain more deeply than that of the surrounding cells.

Oppel, 97, described, in the glands of Lieberkühn of Echidna, cells, at the bottom of the gland, the inner segment of which was finely granular. The granules diminished in number as the mouth of the gland was approached and in the upper portion of it were wholly lacking.

Schmidt, 05, studied the distribution of the cells of Paneth in the human intestine and confirmed the observations of Bloch, 03, who found them in practically every gland of the ileum and jejunum, as well as of the duodenum. In addition, Schmidt found Paneth cells frequently present in the glands of the vermiform appendix, although he was not able to find them in other portions of the large intestine except in three cases of pathological conditions. Concerning the occurrence of Paneth cells in the large intestine of the infant where Bloch claims to have observed them, Schmidt records a negative result in five newborn children.

For the differentiation of goblet cells from Paneth cells Schmidt used mucicarmine by means of which he obtained a sharp distinction even in the foetal intestine. As far as the function of the Paneth cells is concerned he regards the fact of their absence from the intestines of even young carnivora as opposed to the conclusion which might be drawn from Bloch's observation of their occurrence in large numbers in the large intestine of suckling infants, that they have something to do with the secretion of a substance which is active in the digestion of milk. He is rather inclined to the view that inasmuch as they are constantly present in the glands of herbivorous animals they affect some constituent of the vegetable food.

As far as the occurrence of Paneth granule cells in lower classes of Vertebrata is concerned comparatively few references can be found in the literature. Nicolas, 91, in the article already referred to mentions their occurrence in the lizard without stating the species examined, and E. Bizzozero, 04, has described, in the depressions between the folds of the intestine in Teleostomes, cells which contain numerous granules stainable in haematoxylin but differing in their characters from the young goblet cells which occur in the same location.

The last decade has been particularly fruitful in researches dealing with the morphology and microchemistry of glandular cells. As a result of these, new methods have been devised and new criteria established for distinguishing between zymogenic cells and mucous cells. In particular may be mentioned in this connection the basal filaments of Solger, 94, which have been shown by the researches of Bensley, 96, Garnier, 00, Cade, 00, Zimmermann, 98, and others to be a structure common to many sero-zymogenic cells. Bensley, 96, 98, has also shown that the basal filaments correspond to the chromatin of the nucleus in their staining reactions and like the latter contain iron in the form of an organic compound and thus represent morphologically the substance presumably of nuclear origin which Macallum, 95, long ago discovered in gland cells of various sorts by means of staining reactions, and subsequently confirmed by means of the microchemical reaction for iron. As far as mucin is concerned no microchemical reaction has been, as yet, discovered, which is effective in recognizing this substance in isolated cells in sections. The work of P. Mayer, 97, has, however, provided us with a number of new staining solutions which, while they do not permit us to say whether a given cell does or does not secrete mucin, yet furnishes evidence which may be of much value when taken in connection with that from other sources.

Up to the present no special attention has been directed to the question of the presence of Macallum's prozymogen in the cells of Paneth, either in the form of basal filaments or as a diffused compound in the base of the cell, although several observers, notably Zipkin, 04, and Nicolas, have called attention to the deeper staining of the protoplasm of these cells as compared with neighboring cells. The results, moreover, of attempts to discover experimentally, differences in the aspect of these cells corresponding with phases of physiological activity have not been decisive. Accordingly, at the suggestion of Professor Bensley I undertook the reinvestigation of these structures in the hope that the application of new staining and microchemical methods would reveal new facts which would be of assistance in forming an opinion as to their nature and their relationship to other intestinal epithelial elements.

At a very early stage in the investigation the discovery was made that in the opossum, Didelphys virginiana, the cells of Paneth occurred not only in the glands of Lieberkühn but also mingled with other epithelial elements on the sides of the intestinal villi even at their very tips. This remarkable fact, which possesses no parallel in any other mammal, so far as is known, possesses so much significance in the interpretation of the Paneth cells that a somewhat extended description is called for.

The small intestine of the opossum is characterized by extremely long villi and a very thin tunica mucosa. Corresponding to the latter the glands of Lieberkühn are very short and contain a scarcely recognizable

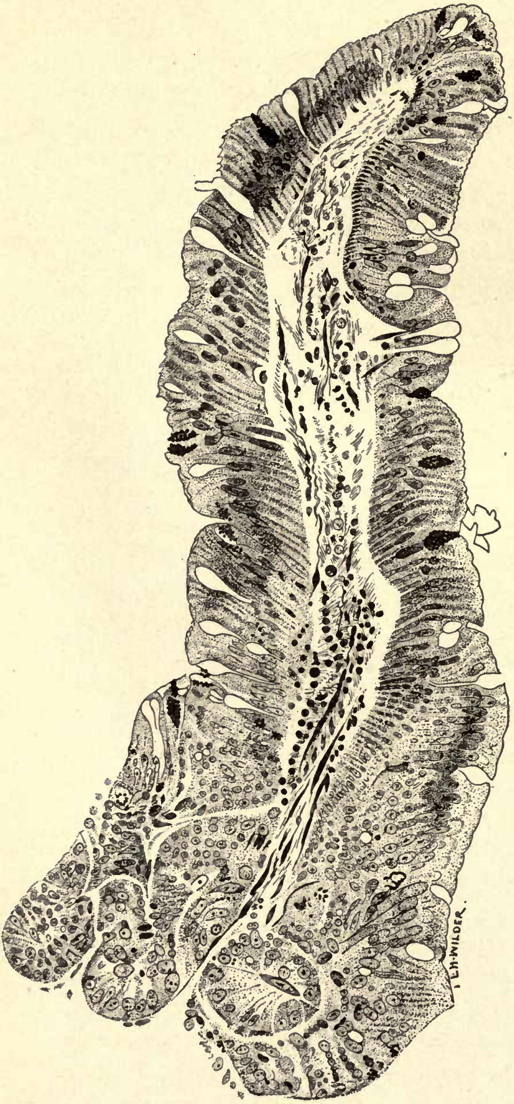


FIG. 1.

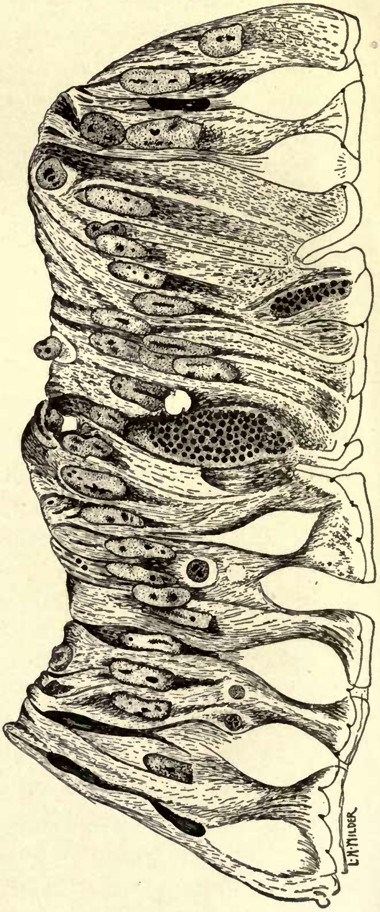


FIG. 2.

FIG. 1. Villus and subjacent glands of Lieberkühn of the opossum. From a preparation stained in iron-alum hematoxylin and mucicarmine.

FIG. 2. A portion of the epithelium of the villus shown in Fig. 1 as seen under Leitz Homog. Imm. 1/12, Oc. 4.

lumen. Fig. 1 illustrates fairly well the nature and distribution of the three kinds of epithelial cells in the glands and in the villi. The preparation is from material fixed in Bensley's bichromate-sublimate-alcohol fluid and stained with iron haematoxylin followed by mucicarmine. By this means the granules are stained blue-black in the cells of Paneth, red in the goblet cells. Thus a sharp differentiation is obtained between these cells even in the glands of Lieberkühn where the young cells contain comparatively little of the secretion-antecedent. The Paneth cells on the side of the villus are large and resemble very closely typical goblet cells (fig. 2). The theca is filled with large discrete granules which do not react with mucicarmine but on the contrary stain intensely in iron haematoxylin. These granules also stain strongly in the neutral gentian mixture recommended by Bensley, 02, for staining zymogen granules, the mucous goblet cells remaining colorless. The granules in the Paneth cells occupy the meshes of a network which is formed by the cytoplasm separating the granules. At the proximal end these cells are narrower and contain a nucleus which is somewhat elongated in the direction of the long axis of the cell and often slightly cupped on the side next the theca. The basal protoplasm is small in amount and uniformly more deeply stained than that of neighboring cells, with the exception of the narrow cells which are obviously undergoing degeneration. Tested with Macallum's reagents for the detection of organic iron a positive result is obtained but not enough to be convincing evidence of the presence of prozymogen in the cell. The search for basal filaments also proved without result although the positive outcome of these observations in the guinea-pig, to be described presently, gave ground for the belief that had a more abundant material been available, and had it been possible to examine it in different physiological states, a positive result might have been obtained.

The glands of Lieberkühn in the opossum are remarkable for their low grade of development, and, although the three main types of cells are present, the amount of secretion which the Paneth cells and goblet cells contain indicates that they are to be regarded rather as young elements than as cells already functioning as secreting organs. Indeed, in some respects, these glands present but little advance over the epithelial buds to be found in the intestinal epithelium of Batrachia, and to this extent realize Bizzozero's idea of a gland of Lieberkühn which serves merely as a place for the production of new cells which ultimately migrate to the free surface and there reach their full functional development. Many mitoses are always present in the glands, some in mucinholding cells, others in the cylindrical cells. I have not been able to assure myself definitely of the occurrence of mitoses in the cells of Paneth. The small size of these cells and their more irregular shape and arrangement make the exact determination of the nature of the mitotic cells somewhat difficult.

The distribution of the cells of Paneth in the intestinal epithelium of the opossum, the occurrence of the fully loaded cells in the surface epithelium and of immature cells in the gland cannot be reconciled in any way with Bizzozero's view that they are young cells which only achieve their full development as mucus-secreting goblet cells, nor, indeed, with any view except that they are specific elements engaged in the production of a special secretion.

The material from the guinea pig proved the most fruitful in results as regards the cytological characters of the cells of Paneth and in this animal results were obtained which bring the cells of Paneth into line with other sero-zymogenic cells such as the cells of the parotid gland, the chief cells of the fundus glands and the pancreatic cell.

At first, considerable difficulty was experienced in obtaining accurate fixation of the granules. Aqueous sublimate, Bensley's alcohol-bichromate-bichloride mixture, and Kopsch's formaline bichromate mixture, were tried with only partial success. A few of the cells at the very edge of the section, in these imperfect fixations, would be found to have retained the granules while from the majority of the cells they had either been removed entirely or only retained in an imperfect and distorted form. Very frequent in these cases were the crescent-shaped granules described by Nicolas in the Paneth cells as one of the stages in the secretory history of the cells. A great deal of the work was done on material fixed in 10 per cent formaldehyde which penetrated somewhat better than the other fluids mentioned above, although in this fixing fluid the crescentic-shaped granule was common. When the work was nearly completed we succeeded in obtaining complete fixation of the granules by means of a combination of equal parts of alcoholic sublimate and Kopsch's fluid. In preparations fixed in this mixture the granules retained their round form and were perfectly fixed in all the cells of the material.

For staining, Bensley's neutral gentian was employed with good success to differentiate Paneth cells, the granules of which stained intensely violet, from goblet cells which remain colorless or faintly violet. Another method which has rendered great service is staining in iron haematoxylin followed by mucicarmine. By this method the granules of the Paneth cells are stained deep blue-black, those of the goblet cells carmine red.

To demonstrate prozymogen as basal filaments or as a diffuse chromophile substance in the base of the cell toluidene blue was employed in saturated aqueous solution. Better results, however, were obtained by a method devised by Bensley of staining in toluidene blue, orange G, and acid rubin. This method is as follows: the sections cut as thin as possible in paraffin and fastened to the slide by the water method are passed through benzole, absolute alcohol, and graded alcohols, to water. They are then stained for a period of one minute with a mixture containing equal parts of the saturated aqueous solutions of Orange G, and acid rubin. Then wash in water and stain for one minute in saturated aqueous solution of toluidene blue. Wash in water; transfer to absolute alcohol; clear in benzole, and mount in balsam. The result as far as the distribution of the toluidene blue is concerned is much the same as that obtained by staining with this dye alone. The intensity of the blue stain, however, is much increased, and in addition the method offers the advantage of the contrast stain produced by the rubin and orange. By this method chromatin and prozymogen (or basal filaments) are stained intensely blue, protoplasm faint bluish, zymogen granules red, and the contents of goblet cells remain unstained.

Confirmatory evidence of the presence of prozymogen in the Paneth cells was sought by means of the microchemical reaction for organic iron introduced by Macallum.

In the guinea pig Paneth cells are very abundant in the glands of Lieberkühn of the small intestine. They occupy chiefly the deep ends of the gland where they often form a continuous layer which is interrupted by comparatively few goblet cells. A few also occur on the sides of the gland but the upper ends of the glands are wholly free from them.

The structure of these cells depends on the stage of physological activity. In the animals which are kept constantly supplied with food of which they are allowed to partake at will, the cells are cylindrical in shape, the outer end being somewhat broader than that which is directed towards the lumen. In each cell two zones of about equal width are easily recognized. The distal zone directed towards the lumen of the gland is occupied by fine granules which are so closely crowded that it is often difficult to recognize the thin laminae of cell-protoplasm which separate them from one another. In material fixed in aqueous sublimate, however, many cells may be found from which the granules have been removed and here we find the distal zone occupied by a fine meshwork which corresponds in the size of its spaces to that of the granules, indicating that each granule occupies a small space in the protoplasm, a thin lamina of which separates it more or less completely from its neighbors. The granules stain intensely in iron hematoxylin and in neutral gentian but remain quite unaffected by muchaematein or mucicarmine. The proximal or basal zone of the cell contains an oval nucleus which is surrounded by a small quantity of protoplasm which takes a slightly deeper stain than that of neighboring cylindrical cells. Some of the cells contain a larger quantity of this basal protoplasm and in a few of the cells this exhibits a distinct radial striation, in which case the deeper stain is largely confined to the striae. The presence of these basal striations was, however, more easily demonstrated in those animals which were protected from an excess of physiological stimulation by controlling the amount of food taken and supplying it at regular intervals. Fig. 3 represents the lower end of the gland from the small intestine of a guinea pig which had fasted for twenty-four hours after receiving a mixed meal of carrots and oats. The aspect of the cells in this case is very different from that seen in the animal feeding irregularly. In the first place the granules are more than twice as large, and there appears to be a larger number, although, for obvious reasons, it is difficult to be sure of this. In sections of the intestine stained with toluidene blue alone, or with

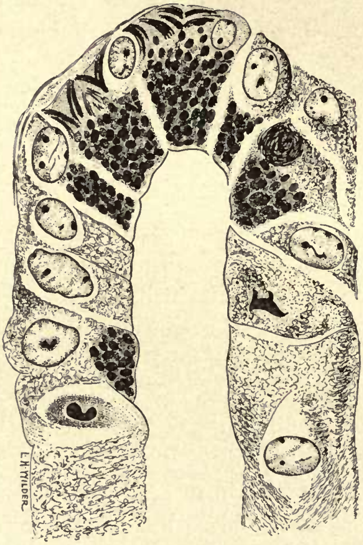


Fig. 3. Bottom of gland of Lieberkühn of the guinea-pig after twenty-four-hour fast. From a preparation stained by orange-rubin-toluidene blue method. Leitz 1/12, Oc. 4.

toluidene blue and orange-rubin, the basal cytoplasm of practically every cell exhibits a radial striation which is exactly similar to that described in various sero-zymogenic gland cells by Bensley, Solger, Garnier and others. This character is well illustrated in fig. 3 which is from a specimen stained in the toluidene blue-orange-rubin method. The basal filaments stain intensely in toluidene blue, less intensely in iron haematoxylin, but may be observed without difficulty in sections stained with alum-haematein. The most effective method of demonstrating the basal filaments is by means of the microchemical reaction for organic iron of Macallum, because the result is not confused by the faint protoplasmic stain which is obtained generally in staining with toluidene blue. This method consists in liberating the iron from its organic combinations by treatment of sections from material hardened in alcohol with a solution of sulphuric acid in alcohol for several hours at 37.5 C. and then demonstrating the iron at the point of its liberation by means of haematoxylin (see Macallum, 95). The result, as far as the Paneth cells are concerned, is a strong reaction in the substance of the basal filaments and in the nuclear chromatin (fig. 4). In some of the Paneth cells a more

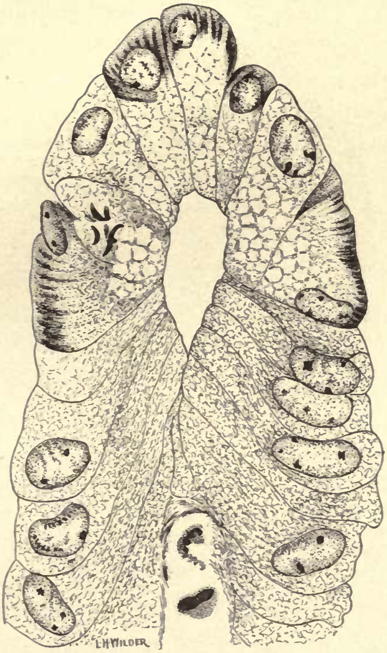


Fig. 4. Gland of Lieberkühn of guinea-pig. From preparation treated by Macallum's method for the detection of masked iron; Leitz 1/12, Oc. 4.

diffuse reaction is obtained indicating that the specific substance on which the staining reaction of the basal filaments depends is present although not definitely organized in the form of filaments.

The absence of the basal filaments from the majority of the cells of the animal which has been kept constantly supplied with food is doubtless due to the fact that in this case the cells are subjected to a physiological stimulus which is practically continuous, which results in a constant drain on the reserve substances of the secretion, both the granular zymogen and its antecedent prozymogen.

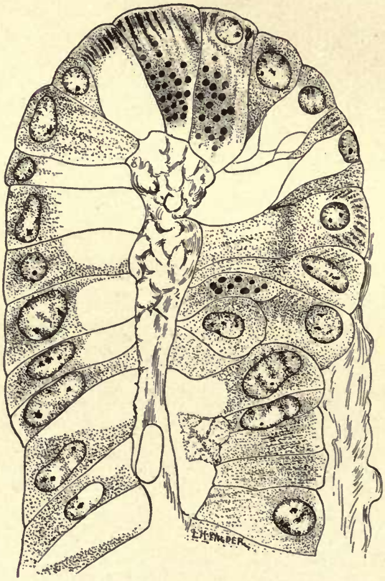


Fig. 5. End of gland of Lieberkühn of guinea-pig six hours after food. Preparation stained in iron-alum hematoxylin and mucicarmine; Leitz 1/12, Oc. 4.

In order to determine the effect of physiological stimulation on the Paneth cells I have examined them in sections taken at gradually increasing intervals after feeding, from animals which have previously fasted for twenty-four hours in order to bring the cells to the condition of maximum loading. The results of these experiments show beyond a a doubt that the cells of Paneth respond to the stimulus of food, although considerable differences were found in the rate of disappearance of the granules in different sets of experiments. The condition of the cell in animals which have fasted for twenty-four hours has been already described and figured (Fig. 3). As early as four hours after feeding, a change in the number and size of the granules may be noticed. They are obviously reduced in number and are somewhat smaller. At six hours the condition represented in Fig. 5 is presented. The cells are longer and narrower and the granules are small, few in number, and occupy the distal segment of the cells only. Indeed, at this stage the cells have assumed very much the same appearance as they present in the animal which is feeding irregularly, although the amount of prozymogen is greater in the six hour stage. At no stage of secretion have I found the fusion of granules into large masses described by Nicolas, if a fixative was employed which was effective in fixing the granules in all parts of the tissue. With some of the fixatives mentioned at the beginning of this paper great differences were found in granules in different cells both in staining power and in form. Very common in these imperfect fixations were the granules composed of a faintly stained central mass with a deeply staining crescent at one border corresponding to the safraninophilous body of Nicolas. I have no doubt that these appearances are entirely due to imperfect fixation, and that the crescent-shaped granules have no existence in the living cell. On the other hand, the different behavior of granules towards the same fixative even in the same cell indicates clearly that some of the granules differ from others either chemically or physically, and it is probable that this difference is due, as Nicolas supposed, to a change in the granule preparatory to its solution and extrusion from the cell as a part of the secretion.

As regards the prozymogen the experiments did not result in a great change in its amount. It is probable that an equilibrium is established between the rate of production and use of this substance which results in the amount in the cell being kept fairly constant.

Summary of Results.

The results recorded show clearly that the cells of Paneth correspond in their structure and microchemical reactions to the enzyme-producing cells of other granular organs, such, for example, as the cells of the parotid gland, the chief of the fundus glands of the stomach, and the cells of the pancreas.

In common with these cells the cells of Paneth contain granules which do not stain in the specific stains for mucin, such as mucicarmine and muchamatein, and which react like zymogen granules to such stains as iron hematoxylin, neutral gentian and acid rubin. In addition they contain in their basal segment a substance which is distinguished by its affinity for basic dyes, such as toluidene blue, sometimes as a diffused substance in the base of the cell, generally in the form of basal filaments. The failure of previous observers to find this substance is probably to be explained by the fact that the study of these cells has generally been undertaken in herbivorous animals which like the guinea pig show the effects of an almost continuous secretory activity. Furthermore, this basal substance, whether diffused in the basal cytoplasm or in the form of the so-called basal filaments, gives when treated by Macallum's method a decided reaction for iron. For these reasons it seems certain that we have to do here with a substance exactly comparable to the prozymogen of other zymogenic cells.

In the opossum the cells of Paneth are found not only in the glands of Lieberkühn, but also on the surface of the mucous membrane. Indeed, when the small size of the cells in the glands, their large size on the villi, and the generally rudimentary character of the glands in this animal are taken into consideration, it seems probable that the cells are formed in the glands, but only reach physiological maturity after migrating to the surface in the way described by Bizzozero. This is of course only true, as far as we know at present, of the opossum, although it is possible that the examination of other polyprotodont marsupials might reveal similar conditions in them. In placentals the cells of Paneth appear to be confined to the bottoms of the glands of Lieberkühn, which thus function as true glands as maintained by Oppel.

Whether the condition found in the opossum is the primitive condition for mammals or not it is impossible to say, although this view presents many interesting possibilities. Nicolas' observation that they occur as a part of the general intestinal epithelium in a lizard points in this direction. The observations bearing on the occurrence of Paneth cells in lower vertebrates are, however, as yet, too few to enable any opinion to be offered as to their phylogenetic source.

The distribution of the cells of Paneth in the opossum absolutely excludes the possibility of the cells of Paneth being young mucous cells, and equally opposed to this view are the facts brought out by Nicolas, Möller, and the writer, as to the indications of active secretion in the structure of the cells and as to their response to physiological stimulation.

The cells of Paneth of the guinea pig respond to physiological stimulus of food by secretion as indicated by changes in the form of the cell and reduction in the number and size of the granules.

The crescent-shaped granules of Nicolas and others are due to imperfect fixation and have no previous existence in the cell.

It seems clear from these facts that the cells of Paneth are specific elements engaged in the secretion of a special substance, probably an enzyme which is of use in digestion. It is not, however, possible to connect the Paneth cells with the formation of the new substances, secretin, erepsin, and enterokinase, because these substances are also found in the small intestine of carnivora in which Paneth cells do not occur. Nor is it possible to connect them definitely with any particular class of food, as Möller suggests, because they have been found not only in herbivorous and granivorous animals, but also in insectivora.

In conclusion, I wish to express my indebtedness to Professor Bensley, at whose suggestion and under whose direction the work was undertaken, for friendly guidance and advice during the progress of the investigation.

REFERENCES.

Bensley, R. R., 96.—The Histology and Physiology of the Gastric Glands. Proc. Canad. Inst., Toronto, 1896. I, pp. 11-16.

——— 98.—The Structure of the Mammalian Gastric Glands. Quart. J. Micr. Sc., Lond., 1898, N. S. XLI, pp. 361-389.

——— 03.—The Structure of the Glands of Brunner. The Decennial Publications of the University of Chicago, 1903. First Series, X, pp. 279-326.

Bizzozero, E., 04.—Sur la régénération de l'épithélium intestinal chez les poissons. Arch. ital. de biol., Turin, 1904, XLI., pp. 233-245.

Bizzozero, G., 92.—Ueber die schlauchförmigen Drüsen des Magendarmkanals und die Beziehungen ihres Epithels zu dem Oberflächenepithel der Schleimhaut. Arch. f. mikr. Anat., Bonn, 1892, XL., pp. 325-374.

Garnier, C., 00.—Contribution à l'étude de la structure et du fonctionnement des cellules glandulaires séreuses. J. de l'anat et physiol., etc., Par., 1900, Année XXXVI, pp. 22-98.

Macallum, A. B., 91.—Contributions to the Morphology and Physiology of the Cell. Tr. Canad. Inst., Toronto, 1891, I., pp. 247-78.

——— 95.—On the Distribution of Assimilated Compounds of Iron other than Hæmoglobin and Hænatins in Animal and Vegetable Cells. Quart. J. Micr. Sc., Lond., 1895, XXXVIII., pp. 175-274.

Mayer, P., 97.—Ueber Schleimfärbung. Mitth. a. d. zool. Station zu Neapel. Leipz., 1897, XII., pp. 303-30.

Möller, W., 99.—Anatomische Beiträge zur Frage von der Sekretion und Absorption in der Darmschleimhaut. Ztschr. f. wissensch. Zool., Leipz., 1899, LXVI., pp. 69-135.

Nicolas, A., 91.—Recherches sur l'épithélium de l'intestin grêle. Internat. Monatschr. f. Anat. u. Physiol., Leipz., 1891, VIII, pp. 1-62.

Oppel, A., 97.—Lehrbuch der vergleichenden mikroskopischen Anatomie, Jena, 1897, Teil II, Schlund und Darm.

PANETH, J., 88.—Ueber die secernierenden Zellen des Dünndarm-epithels. Arch. f. mikr. Anat., Bonn, 1888, XXXI, pp. 113-191.

SCHAFFER, J., 91.—Beiträge zur Histologie menschlicher Organe. Sitzungsb. d. k. Akad. d. Wissench., Math. -naturw. Cl., Wien, 1891, Vol. C, Abth. III, pp. 440-481.

SCHMIDT, J. E., 05.—Beiträge zur normalen und pathologischen Histologie einiger Zellarten der Schleimhaut des menschlichen Darmkanales. Arch. f. mikr. Anat., Bonn, 1905, LXVI, pp. 12-40.

SCHWALBE, G., 72.—Beiträge zur Kenntniss der Drüsen in den Darwandungen insbesondere der Brunner 'schen Drüsen. Arch. f. mikr. Anat., Bonn, 1872, VIII, pp. 92-140.

SOLGER, B., 94.—Zur Kenntniss der secernierenden Zellen der Glandula submaxillaris des Menschen. Anat. Anz., Jena, 1894, IX, pp. 415-419.

ZIPKIN, R., 04.—Beiträge zur Kenntniss der gröberen und feineren Strukturverhältnisse des Dünndarms von Inuus rhesus. Anat. Hefte, Wiesb., 1904, XXIII, pp. 113-186.

Property of
The Department of Anatomy
Reserve Book Shelf